Title of the invention

METHOD FOR IMPROVING THE FREEZING TOLERANCE OF PLANTS

Field of the invention

The invention relates to a method to increase the cold or freezing tolerance of plants by cold acclimating the plants and/or by treating the same with betaines.

This invention also relates to the inhibition of the growth or the reduction of the growth rate of plants by treating them with betaines.

This invention further relates to the improvement of the germination rate of plant seeds at cold temperatures by treating the same with betaines.

Background of the Invention

Betaine is a non-toxic osmolyte that is thought to play a role in the protection against environmental stresses in particular salinity and drought stress (1, 2). This compound is mostly synthesized in the chloroplast by the enzymes choline monooxygenase and betaine aldehyde dehydrogenase (1). It may accumulate in different cellular compartments to adjust the osmotic balance (3) and increase the stability of protein tertiary structure thus protecting proteins from denaturation (4). *In vitro* studies have shown that betaine can protect membranes of *Beta vulgaris* roots against heat denaturation (5). Several higher plant enzymes were also shown to be protected by betaine from denaturation caused by heat (6) NaCl or KCl (7). Betaine can also stabilize the photosynthetic activity of isolated chloroplasts over time (8) and protect photosystem II against the inhibitory effect of NaCl (9). Interestingly, it was shown that an exogenous application of 25 mM betaine on barley leaves improves recovery after an osmotic stress imposed by polyethylene glycol (-10 bar) (10).

Because betaines have been shown to provide some protection to plants from stressful environmental conditions they have been used to treat soils, plants and seeds.

WO 95/35022 discloses a method for treating seeds with betaine to enhance seedling growth and protect seeds against adverse environmental conditions. The seeds may be soaked and dried or coated with betaine. The adverse conditions enumerated are water stress, excess NaCl, extreme temperature or pH and heavy metal toxicity. What is not taught are the temperature extremes and the benefits with respect to the rate of germination at low temperatures.

WO 96/07320 discloses the application of betaine to improve the yield of grapevines the temperature extremes are between 3°C to 30°C.

In WO 96/41530 and WO 96/41532, different compositions of betaine are disclosed for use in protecting wheat, potato and grapevines against adverse conditions including temperatures between 3°C and 30°C.

These references are silent with regards to freezing temperatures and cold acclimation, and they do not teach the advantage of combining cold acclimation with betaine accumulation in order to improve cold or freeze tolerance.

In France Allard's thesis, it is taught that in the wheat cultivar Fredrick, low temperature exposure for three days at 6°C/2°C (day/night) following the addition of 1000 mM betaine resulted in an initial improvement in survival of the plants as tested by measuring the survival rate at -10°C. The survival rate was shown to be 83%, whereas, when treated with only 1000 mM betaine, the survival rate was 51% when compared to controls. The high amount of betaine used (1000 mM) was shown to have a toxic effect to the plant since it was found that it produces chlorosis of the leaves. In these experiments, the plants did not show complete recovery and died one week after the freezing test. This reference does not teach us the conditions for increasing freezing tolerance, since this experiment did not show an actual increase in freezing tolerance as known in the art. An increase in freezing tolerance is determined by the actual increase of the LT_{50} (temperature at which 50% of the plants are killed). In the case of Allard's, there is no mention about an increase in LT_{50}. It is also worthwhile to mention that the aim of Allard's experiment was not to acclimate the plants but to use low temperature exposure for three days purposely to reduce the growth rate and keep the endogenous level of betaine concentration at a high level. Thus the initial higher survival observed is attributed to the higher betaine content. Cold acclimation is actually defined as a low temperature treatment that results in an increase in freezing tolerance of plants. In Allard's reference, it was clearly stated that three days of low temperature exposure did not result in any increase of freezing tolerance. This reference clearly does not teach us the combined effect of cold acclimation and betaine treatments on freezing tolerance. It further does not teach the effect on cultivars that genotypically exhibit less (lower) freezing tolerance. The decrease in plant growth rate is also described and it is shown to be directly proportional to the amount of betaine administered. When the maximal amount of 1000mM was applied to the cultivar Fredrick, a 29% decreased growth rate occurred when compared to control plants. Finally, in this document there were also teachings relating to the protein WCOR 410. It was taught that the protein WCOR 410 accumulates when plants are subjected to cold-acclimation or by increasing amounts of exogenous betaine. This reference however does not appear to teach the combination of cold-acclimation and betaine on the expression of the *Wcor 410* gene that leads to the improvement of freezing tolerance.

AMENDED SHEET

In the patent publication CA 2,104,142, the present inventors disclose the isolation and sequence of three genes responsive to cold temperature. One of thesegenes is *Wcor410*. However what is not taught is that the protein WCOR410 is induced by betaine in a manner proportional to the amount of betaine applied and that this protein is involved in promoting freezing tolerance in some plants. It does not teach the benefits of combining cold acclimation and betaine administration.

Kishitani et al. (11) and Koster et al. (26) teach the accumulation of betaine in plants during cold acclimation. These references do not teach the combination of an exogenously administered betaine and of cold acclimation, and this for the purpose of improving cold or freeze-tolerance.

Patent publications US 4,360,465 and SU-A-369,887 disclose that betaines are capable of suppressing vertical plant growth at concentrations in the range of 1 μM to 1 mM. Analysis of these documents indicate that betaine was used in the hydrochloride form at a very low concentration to achieve reduced growth. In this case, the growth inhibition was due to the acidity of HCl and not to betaine. Betaine in concentrations of 1 μM to 1 mM has no effect on plant growth. If the authors of the reference SU-A-369,887 had buffered their betaine or used a betaine free base, they would not have observed the claimed effect. In support of this argument, the present inventors observed that all the compounds used in this publication are either the HCl or sulfonium forms (the latter producing sulfuric acid). It is obvious that the observed effect on growth is due to acidic conditions rather than to the betaine compounds themselves. In conclusion, any form of acid solutions would get the same effect and there is no way in which this information could help in predicting that higher betaine concentrations would results in reducing the growth rate of plants.

USP 4,032,325 teaches the herbicide effect of betaines. There is no mention of using betaines for improving cold tolerance.

Statement of the invention

There is now provided a method of increasing cold or freezing tolerance in a plant, which comprises the steps of:

- acclimating said plant to a temperature higher than about 0°C but not lower than the coldest temperature that said plant is capable to withstand, for a time sufficient to induce an optimal cold or freezing tolerance, in said plant, and
- administering betaine or a derivative thereof such as glycine betaine to said plant, in a dosage regimen sufficient to induce the same or different optimal cold or freezing tolerance in said plant;

whereby combined steps of cold-acclimating and administering betaine or derivative thereof increase cold or freezing tolerance of said plant over and above the optimal cold or freezing tolerance induced by each step alone.

Preferably, the dosage regimen does not provide an unacceptable toxicity, more preferably, it is non-toxic to said plant.

Any plant could benefit from such a method, preferably, rosaceae, gramineae and grasses, more preferably, roses, strawberry, golf turf, barley or wheat.

In the two latter plants, the time for cold-acclimating is about four weeks, and the dosage regimen is growing the plants in the presence of a solution of glycine betaine having a concentration lower than about 500 mM, preferably about 250 mM.

In the spring wheat variety Glenlea, in which the optimal freezing tolerance, expressed as the temperature where fifty percent of a plant population die (LT_{50}) is about -8 °C for each step alone, the combined treatment resulted in an increase of freezing tolerance by about 6°C to reach a LT_{50} of about -14°C and further resulted in improving photosynthetic capacity and overall physiology of the plants at cold or freezing temperatures.

The optimal freezing tolerance induced by said each step alone and/or in combination is due at least in part to an increased expression of the gene *Wcor410*.

This invention also relates to the reduction of the growth rate of a plant by at least 30%, which comprises the step of treating the plant with an effective dosage regimen of betaine or derivative thereof which is not lethal, preferably non-toxic to the plant.

When growing the spring wheat variety Glenlea, in the presence of 500 mM of glycine betaine for four days, the growth rate thereof was reduced by about 75%.

Another aspect of the present invention is a method of inhibiting the growth of a plant, which comprises the step of treating said plant with a high dose regimen of betaine or derivative thereof, which may even result in a herbicidal effect.

Another aspect of the present invention is a method of improving the germination rate of plant seeds at a temperature which is higher than about 0°C but

not lower than the coldest temperature that said plant seeds can withstand, which comprises the steps of administering to said seeds an effective dosage regimen of betaine or derivative thereof, and allowing said seeds to germinate at said temperature.

Description of the invention

This invention is described hereinbelow by way of specific embodiments and appended figures, which purpose is to illustrate the invention rather than to limit its scope.

Brief description of figures

Figures 1a) and b). Effect of betaine on FT in the cultivar Glenlea.

Plant survival was determined by the regrowth test as described by Perras and Sarhan (25).

Figure 1a): The survival was evaluated after freezing at -8°C.

Figure 1b): The LT_{50} was evaluated after freezing different samples to various temperatures

NA, 12 day-old control non-acclimated plants; **100, 250,** and **500,** plants treated for 4 days with 100, 250, and 500 mM betaine at 25°C respectively; **CA,** plants cold-acclimated at 6/2°C for 30 days; **CA100** and **CA250;** plants cold acclimated at 6/2°C for 30 days in the presence of 100 and 250 mM betaine respectively. Standard deviation did not exceed ± 10%.

Figures 2a) and b). Effect of betaine on LT_{50} in the cultivar Glenlea

Figure 2a): The freezing test was performed at -8°C.

Figure 2b): The freezing test was performed at -13°C.

NA, 12 day-old control non-acclimated plants; **250** plants treated for 4 days with 250 mM betaine. **CA,** plants cold-acclimated at 6/2°C for 30 days; **CA250;** plants cold acclimated at 6/2°C for 30 days in the presence of 250 mM betaine.

Figure 3. Accumulation of the WCOR410 protein in response to different betaine concentrations in the spring wheat cultivar Glenlea.

Total proteins (5 µg) were separated by SDS-PAGE, transferred to a nitrocellulose membrane and probed with the anti-WCOR410 antibody. NA, 12 day old control non-acclimated plants; 100, 250 and 500, plants treated for 4 days with 100, 250, and 500 mM betaine respectively; CA, Cold acclimated plants.

Figure 4a). Golf turf was treated three times at a weekly interval in fall with 1 L/m^2 of 200 mM betaine free base in water. The control area was sprayed with water only. The figure shows the turf regrowth in following early spring for the non-treated control and the betaine-treated area. The upper view shows the treated area. Notice the rapidity

of regrowth and greening in the betaine-treated area. This result reflects a greater and healthier winter survival of betaine-treated turf.

Figure 4b). Close up caption showing the betaine-treated area (upper view) and the non-treated control (bottom).

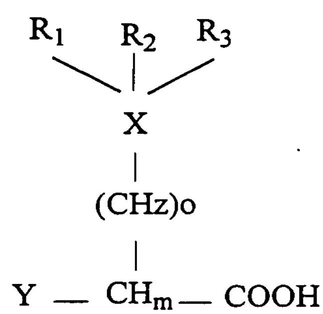
Figures 5a) and b). Another example of the effect of betaine on winter survival and regrowth in early spring. The betaine treatment was the same as in Figure 4 except that the application was started earlier in previous fall. Both areas were aerated at the same time. Notice the higher turf density of the betaine-treated area.

Figure 5a). Betaine-treated.

Figure 5b). Control sprayed with water.

Betaine usually refers to amino acids where the nitrogen is fully or partly methylated. Betaines are natural products present in plants and animals with a probable function as an osmolyte regulator that protect the cell from osmotic stress.

Betaines and derivatives thereof have the general formula:



wherein X is N or S

z is 1 or 2

o is 0 or 1

m is 1 or 2

R_1, R_2, R_3 are independently void or hydrogen or methyl

Y is void or Q-CH_w-

wherein w is 1 or 2,

Q is a molecule of 2 to 9 carbon atoms comprising or not a ketone or a hydroxyl group, which may comprise 1 or 2 nitrogen or sulfur atoms and which may form a heterocycle alone or with X.

Many different betaines are known and some examples are presented in Table 1.

TABLE I

NAMES	OTHER NAMES
Glycinebetaine	Oxyneurin, betaine
β-alaninebetaine	Homobetaine
2-trimethylamino-6-ketoheptanoate	
Prolinebetaine	Stachydrine
L-Proline	
Trans-4-hydroxy-N-methyl-L-proline	
Cis-3-hydroxy-N-methyl-L-proline	
(-)4-hydroxyproline betaine	Betonicine
(+)4-hydroxyprolinebetaine	Turicine
3-hydroxyprolinebetaine	3-oxystachydrine
Histidinebetaine	Herzynine, Ercinine
Tryptophanbetaine	Hypaphorine
2-mercaptohistidine-betaine	Ergothioneine
Pipecolabetaine	Homostachydrine
Nicotinic acid betaine	Trigonelline

Using two wheat cultivars that differ in their levels of freezing tolerance (FT),the role of endogenous betaine was investigated during cold acclimation. In addition, studies on the effect of an exogenous application of betaine on FT alone and in combination with cold acclimation, on the expression of low temperature-responsive genes and on photosynthetic activity have been conducted.

To determine if betaine accumulation is associated with increased FT, the betaine contents were determined in two wheat varieties differing in their FT (cv Glenlea, LT_{50} (lethal temperature for 50% of the plants) of -8°C and cv Fredrick (LT_{50} of -17°C). In both cultivars, betaine content decreases during growth at the non-acclimated temperature of 24/20°C while it increases during growth at the cold-acclimating conditions of 6/2°C. The basal betaine level is 30% higher in the more tolerant cultivar Fredrick before cold acclimation (8.5 µmol/g FW (fresh weight) in Fredrick compared to 6.5 µmol/g FW in Glenlea). At the end of the acclimation period (where maximal LT_{50} has been reached) cv Fredrick has accumulated 21.3 µmol/g FW of betaine compared to 15.3 µmol/g FW for cv Glenlea. On a dry weight basis, cv Fredrick has accumulated 106.5 µmol/g DW (dry weight) compared to Glenlea which has accumulated 82.7 µmol/g DW. This result suggests that the increase in betaine content is associated with the development of FT of the two cultivars. A similar increase in betaine was correlated with the FT of different barley cultivars (11). If we calculate the contribution of betaine to the total osmolality of the cell, we find that betaine accounts for only 3.6% and 4.5% of the osmolality after 30 days of cold

acclimation for Glenlea and Fredrick respectively. This result demonstrates that betaine contribution to the total osmolality is very low. However, as suggested by Wyn Jones *et al* (12), such a low concentration would require compartmentation in order to play a significant role as osmoprotectant. Studies performed by Matho *et al* (13) have shown that betaine is excluded from vacuoles of spinach leaf cells and is mostly found in the cytoplasm and chloroplasts. It was estimated that betaine concentration can reach 300 mM in spinach (14) and *Sueda* (8) chloroplasts when plants are submitted to salt stress. This concentration is approximately 20 fold greater than the average betaine leaf concentration. Betaine compartmentation was not determined in wheat but if we consider a similar concentration factor in the chloroplasts during cold acclimation, the actual concentration of betaine could be very significant. Since we have estimated that betaine accounts for 4.5% of the osmolality in cold-acclimated Fredrick, a twenty fold higher concentration of betaine in the chloroplast would mean that betaine contributes for approximately 90% of the chloroplasts' osmolality (or 612 mOsm). Such a

concentration could have a great impact on chloroplast function since *in vitro* studies have shown that betaine can increase the thermal stability of photosystem II (PSII; (5)) and can protect against the inhibitory effect of NaCl (9). Krall *et al* (16) have shown that betaine can stabilize the active tetrameric form of phosphoenolpyruvate carboxylase which normally forms inactive dimers when exposed to low temperature. Betaine accumulation in the chloroplasts may be an important factor that could play a significant role in maintaining chloroplast function at low temperature. It is worth noting that hardy cereals such as rye, wheat, and barley have higher basal levels of betaine compared to sensitive species such as rice, millet, and sorghum (11).

To determine whether exogenous betaine could play a role in improving FT and photosynthesis at low temperature, we first evaluated the plant's capacity to accumulate betaine. In the first experiment, we incubated plants in 500 mM betaine and determined the osmolality of the leaves at different periods. The osmolality was found to increase rapidly during the first two days and levelled off thereafter (result not shown). We repeated the experiment using different concentrations of betaine and quantified the amount of betaine accumulated in the leaves after a four day period. The method described in (17) was used to extract betaine from 1 g of leaf tissue. Quantitation was performed according to Lerma *et al* (18). For accurate evaluation, an internal standard was added before the extraction procedure. The betaine content was expressed in mOsm/kg H_2O considering the tissue water content for each sample (an average of 82% water content was obtained). Osmolality was measured from leaf tissue after grinding with a mortar and pestle. The liquid obtained was centrifuged at 12,000 *g* for 10 min at 4°C. The osmolality was evaluated in the supernatant using a Wide Range Osmometer. We found that betaine accumulated efficiently at all concentrations used. The accumulated betaine (expressed in mOsm/Kg H_2O) was equivalent to 62% of the external betaine when exposed to betaine concentrations ranging from 118 to 590 mM (100 to 500 mM). Betaine could accumulate even more at higher concentrations, however, signs of chlorosis at the leaf tips became evident at 500 mM. Chlorosis became even more extensive when higher betaine concentrations were used.

Betaine accumulation reduced the growth rate in a manner proportional to the amount of exogenous betaine applied. At the highest concentration used, the growth was reduced by 75% over the 4 day incubation period compared to control plants. The reduction in growth and more importantly, the increase in cellular betaine content was found to be associated with a substantial increase in survival rate after freezing compared to control non-acclimated plants (Fig. 1A). Interestingly, both cultivars are protected by betaine with only a slight advantage in the more tolerant cultivar at all concentrations used (not shown). Plant survival is increased even when a relatively low concentration of betaine is used. At 100mM, survival improved by 5-6 fold compared

to the untreated plants (Fig. 1A). Treating with 250 mM betaine alone was sufficient to increase the FT of the spring cultivar Glenlea from -3°C to -8°C. This value corresponds to the maximal FT achieved by this cultivar after 4 weeks of cold acclimation (Fig. 1B). Increasing the concentration of betaine to a higher concentration resulted in a slightly higher survival rate (corresponding to 55% survival at 500 mM betaine; Fig. 1A) but due to the toxicity, of higher betaine concentrations, the latter were eliminated in other experiments. We have examined whether treatment with betaine during cold acclimation could improve FT in the less tolerant cultivar Glenlea submitted to cold-acclimating conditions. Figs. 1A and 1B show that the survival of plants treated with betaine during cold acclimation were dramatically improved over plants that are cold acclimated in the absence of betaine. Fig. 2 shows the results of a typical experiment for plants treated with betaine at 25°C or during cold acclimation. Betaine treatment at 25°C for 4 days allowed the plants to reach an LT_{50} of -8°C (the maximal LT_{50} normally achieved by this cultivar) while those treated with betaine during cold acclimation were barely affected by a temperature of -13°C (the average LT_{50} was estimated as -14°C in Fig. 1B). These results demonstrate that the improvement in FT observed in control plants exposed to betaine is additive in cold-acclimated plants. This finding is of crucial importance since it is the first time that the normal genotypic potential to tolerate freezing has been improved so dramatically. We have also evaluated the capacity of betaine to improve FT in barley which was also shown to accumulate betaine upon cold acclimation and found that the combined treatment of low temperature and betaine was as efficient in this species as in wheat to improve FT.

It is therefore expected that the improvement in cold or freezing tolerance will be observed in almost all plants, particularly gramineae or grasses, more particularly cereals such as rice, corn, rye, wheat, barley and oat. The conditions at which such improvement will occur are set as follows. The plants are acclimated at the coldest temperature that they can withstand. Betaine is administered before, during and at the end of the cold acclimation. The dosage regimen of betaine is determined on test plants in order to evaluate the doses at which betaine is toxic, in such a way that unacceptable toxic doses will be further avoided. We expect that even tropical plants which are very cold-sensitive will benefit form a combined treatment.

Furthermore, since the growth rate of the plants were reduced down to 25% the control plants, it is readily apparent that, if betaine is applied at the end of cold acclimation (in field, that would mean during fall wherein the maximal growth is attained), this effect would not be deleterious to the plants. Moreover, this property may be advantageously used at the beginning or during the growing period, to slow down the growth of many plants. Thus, it could be used as a growth-retarding substance for several agronomical applications. A specific useful application would be found for golf courses to reduce the maintenance costs (it would reduce the number

of times one has to mow the grass during a season). At higher toxic or lethal doses, betaine could event be used as a herbicide to inhibit the growth of undesirable plants or kill them.

Treatments with other osmolytes such as NaCl or mannitol allowed the osmolality to increase as much as with betaine treatment. However, the FT was not significantly improved when these osmolytes were used indicating that betaine specifically improves FT. These results suggest that the improvement in LT_{50} of 6°C cannot be explained solely by the osmolyte role of betaine (at a concentration of 250 mM, betaine would depress the freezing point of water by 0.32°C). We thus investigated whether betaine can induce a number of genes known to be associated with the development of FT. We examined the expression of three different proteins induced by low temperature using specific antibodies and immunoblot analysis as well as the expression of three other low temperature-induced genes using northern analysis. Our results showed that the protein WCOR410 (Genbank accession no. L29152) accumulates in a concentration-dependent manner when plants are exposed to varying amounts of betaine (Figure 3). When we examined the expression of the WCS120 (Genbank accession no. M93342) protein family or of the WCS19 (Genbank accession no. L13437) protein, we found that these proteins did not accumulate upon exposure to betaine (not shown). Since these two genes are known to be specifically induced by low temperature and that the gene *WCOR410* was inducible by low temperature, salinity, and water stress (19), we examined the possibility that other genes induced by drought or salinity are also induced by the presence of betaine. The genes *WCOR80* (Genbank accession no. U73212), *WCOR413* (Genbank accession no. U73216), and *WCOR825* (Genbank accession no. U73215) were only slightly inducible by betaine (not shown). These results suggest that the *WCOR410* gene is specifically induced to high levels by betaine exposure and may be a major contributor to the improvement of FT above the level that could be explained solely by the role of betaine as an osmolyte. The WCOR410 protein was found to be associated with the plasma membrane. This is the first abundant protein found in the membrane fraction of the vascular tissues (20), a region of the seedling known to be the most freezing sensitive tissue in wheat (21).

Betaine was shown to protect thylakoid membranes from freezing stress *in vitro* (22). Furthermore, in glycine-betaine deficient maize lines, high temperature decreases membrane stability and the resistance to photoinhibition as well as the steady-state yield of electron transport over PSII (23). These results suggest that betaine may confer greater membrane stability under both low and high temperature. It can also protect the photosystem II against salt stress *in vitro*. Since betaine is normally synthesized in the chloroplast, it may accumulate to a higher concentration

in this organelle as suggested (8, 14). Thus, we evaluated the effect of the exogenous supply of betaine on the resistance to photoinhibition and oxygen evolution.

During steady-state photosynthesis at the prevailing growth conditions, exposure of spring and winter wheat to 250 mM betaine resulted in small but consistently higher levels of qP and higher yields of PSII electron transport (Φe) than non-treated controls (Table I). Thus, betaine treated spring and winter wheat seedlings appeared to exhibit a greater capacity to prevent the reduction of PSII reaction centres than non-treated controls. The increased capacity to keep PSII reaction centres oxidized was correlated with a decreased susceptibility to low temperature (5°C) photoinhibition (measured according to 24) in betaine-treated seedlings. The Fv/Fm ratio of 250 mM betaine-treated plants submitted to high light (2h at 5°C and 1600 μmol $m^{-2}.s^{-1}$) was of 84 ± 4% of non-photoinhibited control plants compared to 72 ± 2% for plants not treated with betaine. This effect of betaine on the photosynthetic machinery may improve the physiology and performance of the plants at low temperature and thus provide the plants with a greater capacity to use the available energy to develop FT.

Betaine application early in the fall improves the performance of golf turf and consequently increased winter survival. The betaine-treated turf showed a rapid regrowth in the spring indicating a higher winter survival rate and healthier plants at spring which have a better regrowth rate.

Based on the above results and based on the partial teachings of WO 95/35022, it is further expected that the germination rate of plant seeds can be improved at cold temperature. This method will comprise the steps of administering to the seeds an effective dosage regimen of betaine or derivative thereof, and allowing the same to germinate at cold temperatures. Such cold temperatures would be above about 0°C but not lower than the coldest temperature that the one plant seeds can withstand.

An important conclusion one can draw from these observations is that it may be possible to increase FT by using only one or two of the major genes (such as *Wcor410*) associated with this multigenic trait. Thus, it may be possible to increase the FT of cold sensitive species by transforming plants with this gene. Furthermore, our results suggest that FT could be improved in an additive manner in plants that already possess some degree of FT by overexpressing the most important genes involved in the process of FT. For example, overexpressing betaine dehydrogenase and choline monooxygenase under a low temperature promoter may allow the accumulation of betaine at the time where it could help against freezing stress. Such manipulations of betaine production could have a great agronomic and economic impact not only for protection against drought stress as previously suggested but also for FT as shown in this report. In addition, the results presented in this report indicate that exogenous

application of betaine before a predicted frost or sudden decrease in temperature may be exploited on a short-term basis to increase the resistance of cold sensitive plants to low temperature stress.

This invention has been described in details hereinabove, and it is readily apparent that modifications thereto can be made, without departing from the spirit of the invention and from the above teachings. These modifications fall under the scope of the invention as defined in the appended claims.

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